09/076,115

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ			
L17	L16 and (liga$4 near5 vector$1)	0	L17
L16	L15 and captur$3	0	L16
L15	L14 and cleav$4	5	L15
L14	L13 and reverse	5	L14
L13	primer adapter$1 near5 RNA	11	L13
L12	l10 and RNA	19	L12
L11	l10 and mRNA	19	L11
L10	L9 and (liga$4 near5 vector)	19	L10
L9	l2 and captur$3 and NotI	24	L9
L8	l6 and NotI	0	L8
L7	L6 and (ligat$3 near5 vector$1)	1	L7
L6	L5 and captur$3	14	L6
L5	L4 and reverse transcript$3	44	L5
L4	primer$1 near5 cleav$4 near5 RNA	58	L4
L3	primer adapter$1 near5 cleav$4	0	L3
L2	primer adapter$1	184	L2
DB=DWPI,USPT,EPAB,JPAB; PLUR=YES; OP=ADJ			
L1	primer-adapter near5 cleav$4 site$1	0	L1

END OF SEARCH HISTORY

☐ Generate Collection

L12: Entry 18 of 19 File: USPT Jan 9, 1996

DOCUMENT-IDENTIFIER: US 5482845 A
TITLE: Method for construction of normalized cDNA libraries

Brief Summary Text (11):
Ko (1990) reported the construction of a normalized mouse cDNA library by a complex scheme involving: a) ligation of cDNAs to a linker-primer adapter; b) three rounds of PCR amplification, denaturation-reassociation, and purification of single-stranded cDNAs by hydroxyapatite (HAP) column chromatography; and c) digestion of the end product using a site present in the linker-primer sequence and cloning (#' non-coding cDNA fragments only) into a plasmid vector.

Drawing Description Text (7):
In vitro synthesized RNA from the brain cDNA library (see the text; this library represents all regions of the brain with the exception of hippocampus).
Single-stranded circles from both brain and hippocampus normalized libraries (see the text). Hippocampus specific subtracted library.

Drawing Description Text (8):
FIG. 4 Substractive hybridizations involving RNA from combinations of normalized libraries and single-stranded circles from the cDNA catalogue. In FIG. 4, a spinal cord-specific library is isolated.

Drawing Description Text (9):
In vitro synthesized RNA from all individual normalized libraries (except spinal cord, in this example) will be hybridized to the cDNA catalogue in the form of single-stranded circles. After purification of the remaining single-stranded circles by HAP chromatography and conversion to partially duplex circular molecules for improvement of electroporation efficiencies, the subtracted library can be propagated in bacteria. All clones from this subtracted library should have the sequence identifier of the spinal cord library.

Detailed Description Text (9):
Methods to purify partial duplexes from single-stranded circles have been well-known in the art. In an embodiment, the partial duplexes are purified by hydroxyapatite column chromatography. Other methods such as affinity-capture techniques may be similarly used. One design of the affinity-capture may include using biotinylated deoxynucleotide in the controlled extension reaction and subsequently capture of the incorporated biotinylated nucleotides by avidin conjugated on a column. There are other affinity-capture techniques which may be similarly used in accordance with this invention.

Detailed Description Text (32):
Subtractive hybridization experiments typically involved hybridizing first-strand cDNA (tracer) with an excess of poly (A).sup.+ RNA (driver). The remaining single-stranded cDNAs were separated from the DNA-RNA hybrids by HAP chromatography and either cloned (Travis & Sutcliffe, 1988) or used as a probe in a differential screening procedure (Miller et al., 1987).

Detailed Description Text (33):
Simpler and more efficient methods for subtractive hybridization have now been described. Rubenstein et al. (1991) described a method according to which photobiotinylated single-stranded phagemids from a directionally cloned cDNA library were used as drivers in a hybridization with tracer amounts of complementary single-stranded phagemids. After binding to streptavidin and extracting with phenol:chloroform, the unhybridized single-stranded circles (subtracted library) were recovered from the aqueous phase, converted to partially duplex circular molecules

(for improvement of electroporation efficiencies) and electroporated into bacteria. Swaroop et al. (1991) successfully isolated a number of retina-specific clones by a very simple procedure involving hybridization of in vitro synthesized biotinylated RNA (run-off transcription of a directionally cloned cDNA library in the presence of Bio-11-UTP) with single-stranded phagemids from a directionally cloned cDNA library. DNA-RNA hybrids were captured by affinity to vectrex-avidin (Vector Laboratories) and the single-stranded circles (subtracted library) were eluted, precipitated with glycogen and directly electroporated into bacteria.

Detailed Description Text (39):
The general scheme for construction of directionally cloned cDNA libraries can be outlined as follows: a) a Not I-(dT) 18 oligonucleotide [or Pac I-(dT) 18] is utilized as primer for first strand cDNA synthesis with RNAse H.sup.- Reverse Transcriptase from Moloney Murine Leukemia Virus (Gibco.RTM.-BRL); b) "one tube" first and second strand cDNA syntheses are performed essentially as described (D'Alessio et al., 1987); c) double-stranded cDNAs are polished with T4 DNA Polymerase, size selected on a Bio-Gel A-50 m column as described (Huynh et al., 1985) and ligated to a large excess of adaptor molecules (for the brain library described below which was constructed in the Lafmid BA vector applicants used Hind III adaptors, but for all libraries that applicants are planning to construct in the pT7T3-Pac I vector, applicants will utilize Eco RI adaptors instead); d) cDNAs are treated with T4 Polynucleotide Kinase to phosphorylate the adaptor ends (one of the two oligonucleotides of the adaptor molecule has a 5'OH to prevent concatemerization of adaptors), digested with Not I (or Pac I, depending on the primer utilized for 1st strand cDNA synthesis), size selected again over a BioGel A-50 m column and ligated directionally into the Not I and Hind III (Lafmid BA vector) or Pac I and Eco RI (pT7T3-Pac vector) sites of a phagemid vector; e) the ligation mixture is electroporated into bacteria and propagated under ampicillin selection; f) to eliminate complete from the library all clones that contain inserts shorter than 500 bp, the non-recombinants, and most existing chimeric clones, a plasmid preparation of the library is linearized with Not I (or Pac I), electrophoresed on an agarose gel and the linear recombinant molecules containing cDNAs larger than 500 bp are purified off the gel with .beta.-agarose and recircularized in a large volume ligation reaction; and g) the ligation mixture is electroporated into bacteria and propagated under ampicillin selection to generate a cDNA library with an average size insert of 1.7 kb, no inserts shorter than 500 bp and a very low background of non-recombinant clones.

Detailed Description Text (42):
This problem was solved by strictly size selecting the cDNAs over a 32 cm long-0.2 cm wide BioGel-A50 m column as described (Huynh et al., 1985). Although time consuming, this column is very reliable and reproducible. There are two rounds of size selection; one right after second strand synthesis (before adaptor ligation) and a second after adaptor ligation, just prior to setting up the ligation to the cloning vector. In addition, applicants have introduced a gel purification step after cloning and propagation in bacteria; supercoiled plasmid DNA from the library is linearized by Not I (or Pac I, depending on the primer utilized for first strand cDNA synthesis) digestion, and electrophoresed on a 1% agarose gel; and the DNA smear corresponding to cDNAs with insert sizes larger than 500 bp is cut off the gel, casted into a low melting point agarose gel, and electrophoresed backwards to sharpen the DNA smear. Library DNA is then purified by digestion of the gel slice with .beta.-agarose. Following a ligation reaction performed under conditions that promote recircularization only, the DNA is electroporated into bacteria (DH10B, BRL.RTM.) and propagated under ampicillin selection. The exact reaction conditions to promote recircularization rather than inter-molecular ligations can be determined by the formula 3.3/.sqroot. kb .mu.g/ml as discussed by Smith et al. (1987).

Detailed Description Text (49):
Another step where chimeric clones can be generated is during ligation of the cDNAs to the cloning vector. This is less likely to occur, however, because the cDNA have two different ends and three cDNA molecules must be joined together before they can be ligated to a vector molecule. Nonetheless, in order to minimize the probability of formation of chimeric clones during this ligation reaction, vector should be present in excess over cDNAs. Since dephosphorylation usually reduces cloning efficiencies, the approach of not dephosphorylating the vector and using it in only a slight excess is favored; a twofold excess over cDNAs seems to be a good compromise. Under these

conditions, chimeric clones are unlikely to be formed and the background of non-recombinant clones still remains low.

Detailed Description Text (53):
Some precautions are necessary to avoid non-specific priming at GC-rich regions of the mRNAs when using large amounts of the Not I-(dT)18 primer for first strand cDNA synthesis. Most importantly, the reaction mixture should be pre-incubated at 37.degree. C. before the addition of reverse transcriptase. It was observed that if the enzyme is added to the reaction mixture while it is at room temperature, an appreciable number of clones without tail can be obtained. For example, clones for the mitochondrial 16S rRNA which resulted from priming events at two sites of the RNA sequence that differ from the recognition sequence of the Not I restriction enzyme by a single nucleotide have been obtained. Presumably, if a GC-rich cluster is flanked by a few (A)s located upstream on the RNA, the Not I sequence (GCGGCCGC, SEQ ID No. 1) of the primer can anneal to it while most of the oligo-dT tail loops out. The end product of such non-specific priming events can be a clone without a tail or a clone with a very short tail (shorter than the primer). These clones are easily detected because a bona fide polyadenylation signal sequence (AAUAAA, SEQ ID No. 2) cannot be identified at the appropriate position.

Detailed Description Text (57):
Because the Lafmid BA vector, which was utilized for construction of the infant brain cDNA library described below, does not have the promoters for in vitro synthesis of RNA, applicants decided to switch to another cloning vector (pT7T3, Pharmacia.RTM.). There was no reason to modify the Lafmid BA vector to include RNA promoters since other phagemids are already available that have all features that was needed. Accordingly, the polylinker of the pT7T3 phagemid vector (Pharmacia.RTM.) was modified to include a Pac I site. This modified vector was named pT7T3-Pac by applicants. This vector has all the features that was needed to normalize and subtract libraries, i.e., it has an fl origin for production of single-stranded circles upon super-infection with a helper phage and it contains both the T3 and T7 promoters for in vitro synthesis of RNA.

Detailed Description Text (58):
The sequence of the polylinker of the pT7T3-Pac vector is: ##STR1## The plan is to clone cDNAs directionally into the Eco RI and Pac I sites of this phagemid vector. Accordingly, Pac I will be utilized to linearize the library for the gel purification step. Since (mRNA-like) and antisense RNA can be transcribed in vitro off the existing T7 and T3 promoters, respectively, which immediately flank the polylinker. Single-stranded circles will have the mRNA-like strand. Therefore, run-off transcripts from the T3 promoter will be complementary to the library in the form of single-stranded circles.

Detailed Description Text (73):
The gel slice containing the single-stranded library DNA smear was casted into low melting point agarose. The current was reversed and the low melt agarose gel was run for a short time just to sharpen the smear. DNA was isolated after digestion with .beta.-agarose (NEB). This gel purification step proved to be necessary to avoid undesirable internal priming events promoted by small RNA oligonucleotides (breakdown products from RNAse A digestion of tRNAs). The single-stranded DNA was never exposed to UV light (a small fraction of it was run on a separate lane, which was exposed to UV and served as a reference; this DNA was not used).

Detailed Description Text (76):
In the next step these partially duplex circular molecules were purified from any remaining (unprimed, unextended) single-stranded circles by HAP chromatography (applicants have actually also tried incorporating biotinylated nucleotides during the extension reaction to allow capturing of the partial duplex circles by affinity to streptavidin-coated solid supports; the results were not satisfactory in applicants' hands especially because the procedure resulted in a dramatic impairment of electroporation efficiencies).

Detailed Description Text (94):
Infant brain (total brain from a 3-month old human infant who died in consequence of spinal muscular atrophy). High quality mRNA is available. This mRNA was already

utilized for construction of a cDNA library in the lafmid BA vector. However, because the lafmid BA vector does not allow for in vitro synthesis of RNA, a feature that is required in applicants' strategy for library subtraction, applicants will utilize this mRNA again to construct a cDNA library in the pT7T3-Pac vector.

Detailed Description Text (96):
Adult brain (a collection of tissue samples representing all regions of the brain with the exception of hippocampus). Power was prepared from multiple areas of the brain and pooled. These areas included frontal, parietal, temporal and occipital cortex from the left and right hemispheres, subcortical white matter, basal ganglia, thalamus, cerebellum, midbrain, pons and medulla. High quality RNA is already available.

Detailed Description Text (98):
Adult hippocampus (obtained from the same brain of that utilized for construction of library #1). Both hippocampi were utilized. High quality RNA is already available.

Detailed Description Text (114):
(1) Synthesize biotinylated run-off transcripts with Bio-11-UTP (Enzo Biochem) and then use vectrex-avidin (Vector Laboratories) to capture the hybrids and thereby purify (flow-through) the unhybridized single-stranded circles (tissue-specific sublibrary) which can then be electroporated into bacteria (after conversion to partial duplexes by random priming for improvement of electroporation efficiencies).

Detailed Description Text (115):
(2) Synthesize non biotinylated run-off transcripts and then use HAP column chromatography to separate the remaining single-stranded material (subtracted library, HAP-flow-through) from the RNA-DNA hybrids (HAP-bound).

Detailed Description Text (116):
Supercoiled plasmid DNA from a normalized library will be linearized with Sfi I and in vitro transcribed from the T3 promoter (run-off transcription) to generate large quantities of antisense RNA which will be complementary to any of the directionally cloned normalized libraries in the form of single-stranded circles (mRNA-like strand). The reactions will be performed with the "Riboprobe Gemini II In Vitro Transcription System" (Promega, Cat#P2570), according to the manufacturer's instructions. A cDNA in the pT7T3-Pac vector has been subcloned to test its ability to drive transcription off the T7 and T3 promoters, and very good yields of RNA were obtained in both cases. Very good yields of single-stranded DNA circles with this plasmid (better with M13K07 than with R408) are routinely obtained.

Detailed Description Text (117):
The in vitro synthesized RNA will then be hybridized to single-stranded circles from normalized libraries. However, since the first 15-20 nucleotides at the 5' end of the in vitro synthesized RNA will be complementary to the sequence of the polylinker immediately flanking the Pac I cloning site, precautions need to be taken to prevent hybridization between RNA and single-stranded circles through such sequences. Applicants plan to synthesize a "blocking" oligonucleotide, which will have the same sequence of the single-stranded circles in that region [5'(A).sub.18 NNNNTTAATTAAGCGGCCGCAAGCTTATT 3', SEQ ID No. 26]. Thus, to prevent hybridization through such sequences the RNA will first be annealed to an excess of the blocking oligonucleotide, and then digested with RNAse H, which will eliminate that very sequence form the RNA (RNAse H attacks the RNA strand of a DNA:RNA hybrid). The sample will be digested with RNAse-free DNAse, which will destroy both the excess blocking oligonucleotide and the linearized plasmid DNA template, and then hybridized to the single-stranded circles.

Detailed Description Text (118):
Typically 0.2 .mu.g of a single-stranded DNA will be hybridized to 20 .mu.g of RNA for 72 hours at 42.degree.-45.degree. C. in a 10 .mu.l reaction containing 0.5M Sodium Phosphate pH 7.2, 10 mM EDTA, 0.1% SDS, 50% formamide (Cot of approximately 3,000). The remaining single-stranded circles (normalized tissue-specific sublibrary) will be purified either by HAP chromatography or by affinity (lack of) to vectrex-avidin, as discussed above. Applicants have vast experience with HAP and applicants know for fact that it is very reliable for this kind of purification. Thus, at least this approach is guarantee to work, but nonetheless applicants will compare efficiencies with the

alternate method. After purification, the single-stranded material will be converted to partially duplex DNA by random priming (just as applicants have been routinely doing at the end of the normalization procedure) and electroporated into bacteria for propagation under ampicillin selection.

Detailed Description Text (120):
As a model system for optimization of conditions for subtractive hybridizations involving normalized libraries applicants will isolate hippocampus-specific cDNAs (see FIG. 3). Two normalized libraries will be utilized: adult brain library: a collection of tissue samples representing all regions of the brain with the exception of hippocampus) and adult hippocampus library, obtained from the same brain of that utilized for construction of the adult brain library). In vitro synthesized RNA from the adult brain library (driver) will be hybridized (high Cot) to a mixture of single-stranded circles from both adult brain and adult hippocampus libraries (tracers), and the remaining single-stranded circles (hippocampus-specific normalized sub-library) will be purified as discussed above. The presence in the hybridization of single-stranded circles from adult brain library will serve as a built in control. If completion of hybridization is achieved, no single-stranded circles from adult brain library should remain unhybridized. Verification that the subtracted library really corresponds to hippocampus-specific cDNAs will be straightforward by single-pass sequencing (3' end sequencing with the M13 Universal Primer or with a primer complementary to the T3 promoter) of a number of randomly picked clones from the subtracted library. This will be possible because clones from these two libraries can be discriminated by their specific sequence identifiers. So, if all clones from the subtracted library will indeed have the sequence identifier of the hippocampus library applicants will know that the subtraction worked efficiently and that applicants will have isolated a collection of hippocampus-specific cDNA clones.

Detailed Description Text (123):
Applicants will certainly also sequence about 50 clones from the original mixture of single-stranded circles from adult brain and adult hippocampus libraries (the very mixture that will be used as tracer in the hybridization) to assess relative frequencies of clones from the two libraries (the expectation would be to find each at a frequency of about 50%). Applicants will then first sequence 20 clones from the subtracted library. Depending on the extent to which the ratio of clones from the two libraries deviated from the starting frequency of 50% each, applicants will decide whether or not a second round of hybridization should be performed. If it will be necessary applicants will just make single-stranded circles from the first subtracted sub-library and hybridize it to a large excess (100-fold) of in vitro synthesized RNA from library #1 again. Once the sequence data will indicate purity of the subtracted sublibrary (all hippocampus-specific clones) applicants will go on and sequence up to 100 clones to derive a solid and statistically significant number. Each clone will then be sequenced from both 5' and 3' ends. It is our experience that because the sequence obtained from 5' end of a clone will often correspond to coding information, the chances of identifying homologies through database searches increase rather significantly.

Detailed Description Text (128):
The plan is to utilize as a driver a mixture of in vitro synthesized RNA from all but one of the normalized library components of the cDNA catalogue, in a hybridization where the tracer will be single-stranded circles form the cDNA catalogue (which contains all libraries including that one missing in the driver) [see FIG. 4]. In other words, supercoiled plasmid DNA from each individual normalized library (except one) will be linearized and separately utilized as templates for in vitro synthesis of RNA. After annealing to the blocking oligonucleotide, and digestion with both RNAse H and RNAse-free DNAse, as detailed above, all synthesized RNAs (20-40 .mu.g) will be pooled together and hybridized to trace amounts (0.1 .mu.g) of single-stranded circles from the cDNA catalogue. If hybridization goes to completion, only-single stranded circles from the library missing in the driver should be found in the flow-through (HAP or vectrex-avidin) fraction. Once again, verification of tissue-specificity would be easily accomplished by single-pass sequencing.

Detailed Description Text (131):
Applicants anticipate that such resources will prove valuable for many purposes, e.g. identification of novel tissue and temporal-specific transcripts, chromosomal

localization of differentially expressed genes (by painting chromosomes with pools of clones from tissue-specific sub-libraries; since cDNA inserts are large in our libraries this should be straightforward by fluorescence in situ hybridization). Furthermore, the availability of this cDNA catalogue as a reference library should facilitate ongoing efforts for isolation of chromosome-specific cDNAs, large sequencing of cDNAs, and cloning of disease-causing genes. Methods for identification of transcribed sequences from genomic DNA, such as exon trapping (Duyk et al., 1990; Hamaguchi et al., 1992), exon amplification (Buckler et al., 1991), cDNA selection (Parimoo et al, 1991), and direct selection (Lovett et al., 1991; Morgan et al., 1992) should also benefit from this cDNA catalogue. "Exon amplification" and "exon trapping" are methods that take advantage of RNA splicing to capture expressed sequences from large regions of genomic DNA. "Direct selection" and "cDNA selection" utilize a genomic target DNA, a YAC clone for example, for hybrid selection of cDNAs. At the end either the small exons that were trapped or the short cDNA fragments that were selected need to be utilized to "fish" more informative cDNA clones from high quality cDNA libraries. Applicants also have developed a method for hybrid selection of cDNA clones (as single-stranded circles with filter immobilized genomic DNA, which applicants are utilizing to identify chromosome 13-specific cDNAs (Bonaldo et al., manuscript in preparation). However, to identify as many transcribed sequences as possible from any given region of DNA applicants would have to go through selections with a number of different libraries, as opposed to only one, if the cDNA catalogue were already available.

Detailed Description Text (143):
6. Buckler, A. J., et al. (1991). Exon amplification: a strategy to isolate mammalian genes based on RNA splicing. Proc. Natl. Acad. Sci. U.S.A. 88:4005-4009.

Detailed Description Text (148):
11. Duguid, J. R., et al. (1988). Isolation of cDNAs of scrapie-modulated RNAs by subtractive hybridization of a cDNA library. Proc. Natl. Acad. Sci. U.S.A. 85:5738-5742.

Detailed Description Text (158):
21. Kornberg, A. and Baker, T. A. (1992). RNA-Directed DNA Polymerases: Reverse Transcriptases and Telomerase. In "DNA Replication", 2nd Edition, pp. 217-222. W. H. Freeman and Company, New York.

Detailed Description Text (174):
37. Travis, G. H. et al. (1987). Subtractive cloning of complementary DNAs and analysis of messenger RNAs with regional heterogeneous distributions in primate cortex. Neuropharmacol. 26(7B):845-854.

Detailed Description Text (185):
Applicants have developed a method for normalization of directionally cloned cDNA libraries constructed in phagemid vectors, which is also based on the kinetic principle. Briefly, the method involves annealing of the library in the form of single-stranded circles to a NotI oligo (dT)18 primer, and controlled extensions (200-250 nt) with Klenow in the presence of dNTPs and ddNTPs to generate a cDNA library in the form of partially duplex circular DNA molecules that can then be normalized by the kinetic approach, i.e melting and reannealing to moderate Cot, and purification of the unreassociated single-stranded circles (normalized library) by hydroxyapatite column chromatography. Since the unreassociated material (HAP-flow-through) consists of already cloned cDNAs in the form of single-stranded circles, they can be readily electroporated into bacteria and propagated under appropriate antibiotic selection. This is in contrast to the alternative methods, according to which at the end of the reassociation reaction the remaining single stranded cDNAs need to be amplified by PCR and cloned.

Detailed Description Text (191):
Total cellular RNA from a 73 day old post-natal female human brain was extracted according to a modification (Puissant and Houdebine, 1990) of the procedure described by Chomczynski & Sacchi (1987), and poly (A)+RNA was purified by standard procedures (Sambrook et al., 1989). A detailed description of the protocol utilized for construction of this human infant brain cDNA library has been provided elsewhere (Soares, in press).

Detailed Description Text (196):
HAP-purified single-stranded DNA was then purified from any residual amount of tRNA and from most of the helper phage DNA by agarose gel electrophoresis. The agarose gel slice containing the single-stranded library DNA smear was casted into a low melting point agarose gel, the current was reversed and the DNA was electrophoresed backwards (just to sharpen the smear) until it entered the low melt agarose gel. The low melt gel slice containing the library DNA was digested with-agarose (NEE) and the single-stranded circles were ethanol precipitated. This gel purification step proved to be necessary to avoid undesirable internal priming events promoted by small RNA oligonucleotides (breakdown products from RNAse A digestion of tRNAs). The single-stranded DNA was never exposed to U.V. light [A small fraction of it was run on a separate lane, which was exposed to U.V., and served as a reference; this DNA was not used].

Detailed Description Text (220):
To further document that normalization was successful, 190 cDNA clones were randomly picked from the normalized and single-pass sequenced from both ends (Table 3). Database searches of the public nucleic acid and protein databases revealed that 69% (131/190) of the clones correspond to novel brain expressed sequences: no matches could be identified in the public nucleic acid or protein databases to either their 3' or 5' end sequences. 19% (25/131) of those contained repetitive elements (mostly Alu) in either their 3' or 5' ends. 25% (48/190) of the clones had matches to known human sequences, 50% (24/48) of which to "ESTs to unknown genes". 6% (12/190) of the clones were putatively identified based on similarities to known sequences of other organisms, (mainly Rodent, Drosophilia, yeast or C.elegans). Among those putative gene identifications are an homologue of the yeast prmRNA splicing factor RNA helicase PRP22, a homologue of a Drosophila GTP-binding protein, the homologue of the Drosophila puff specific protein B.times.42, and CDNAs similar to the Streptomyces exfoliatus-20 beta-Hydroxysteroid dehydrogenase, yeast hypothetical 43.3 kd protein, Chines hamster DHFR-coamplified protein mRNA, and Rat plasma membrane Ca2+ ATPase-isoform 2 mRNA.

Generate Collection | Print

Search Results - Record(s) 11 through 19 of 19 returned.

☐ 11. 5830662 . 21 Jun 96; 03 Nov 98. Method for construction of normalized cDNA libraries. Soares; Marcelo B., et al. 435/6; 435/91.1 536/23.1 536/25.4. C12Q001/68 C12P019/34 C07H021/04.

☐ 12. 5744306 . 07 Jun 95; 28 Apr 98. Methods for nucleic acid detection, sequencing, and cloning using exonuclease. Murtagh, Jr.; James J., et al. 435/6; 424/94.1 435/174 435/5 435/91.1 435/91.2 536/23.1 536/24.3 536/24.33 536/26.6. C12Q001/68 C12P019/34 C07H021/02 C07H021/04.

☐ 13. 5714596 . 31 Mar 93; 03 Feb 98. NANBV diagnostics: polynucleotides useful for screening for hepatitis C virus. Houghton; Michael, et al. 536/23.72; 435/5 435/6 435/91.1 435/91.33 436/94 536/23.1 536/24.3 536/25.3 536/25.32. C07H021/04 C12Q001/70 C12Q001/68 C12N015/00.

☐ 14. 5698390 . 15 Sep 94; 16 Dec 97. Hepatitis C immunoassays. Houghton; Michael, et al. 435/5; 436/518 436/820. C12Q001/70 G01N033/576.

☐ 15. 5688669 . 07 Jun 95; 18 Nov 97. Methods for nucleic acid detection, sequencing, and cloning using exonuclease. Murtagh; James J.. 435/91.2; 435/6. C12P019/34 C12N015/10 C12N015/66 C12Q001/68.

☐ 16. 5637685 . 06 Jun 95; 10 Jun 97. Normalized cDNA libraries. Soares; Marcelo B., et al. 536/23.1; 435/6 435/91.1 536/25.4. C07H021/04.

☐ 17. 5518901 . 19 Apr 93; 21 May 96. Methods for adapting nucleic acid for detection, sequencing, and cloning using exonuclease. Murtagh; James J.. 435/91.2; 435/91.5. C12N015/10 C12P019/34.

☑ 18. 5482845 . 24 Sep 93; 09 Jan 96. Method for construction of normalized cDNA libraries. Soares; Marcelo B., et al. 435/91.1; 435/6 536/25.4. C12P019/34.

☐ 19. 5350671 . 09 Aug 93; 27 Sep 94. HCV immunoassays employing C domain antigens. Houghton; Michael, et al. 435/5; 435/6 435/975 436/512 436/518 530/300 530/326 530/327 530/328 530/812 530/826 930/220 930/223. C12G001/70 C12G001/68 A61K037/02 G01N033/543.

Generate Collection | Print

Term	Documents
RNA	56691
RNAS	9396
(10 AND RNA).USPT,JPAB,EPAB,DWPI.	19
(L10 AND RNA).USPT,JPAB,EPAB,DWPI.	19

Previous Page Next Page